THE HAZARD FROM MYCOTOXINS - RECENT AUSTRALIAN EXPERIENCE

W.L. BRYDEN

Summary

During the last decade there has been an increase in the reported occurrence of mycotoxins and mycotoxicoses in Australia. Mycotoxicoses reported for the first time during this period were hyperoestrogenism, feed refusal and vomiting, leukoencephalomalacia, ergotism, bovine hyperthermia, and geeldikkop. There is now a greater understanding of factors that determine mycotoxin production by fungi in the field and in storage and there has been considerable advances in the analysis of these compounds especially the application of ELISA technology. Nevertheless mycotoxicoses in Australia are ill-defined and improperly assessed because of the lack of systematic surveys and inadequate knowledge of the effects of mycotoxins on animal health.

INTRODUCTION

There are some 100,000 species of mould, and though not all are toxigenic, they represent a rich source of interesting substances. Chemists have thus far designated some 300 fungal metabolites as having toxic potential for man and animals (Cole and Cox 1981). Obviously, it is not possible within this paper to try and cover this diverse group of compounds, especially as the vast majority have not been associated with a mycotoxicosis. However during this decade, mycotoxicoses previously not recorded in Australia have been diagnosed. This paper will briefly review the implications of these reports and provide an update on recent advances in mycotoxicology.

MYCOTOXINS IN AUSTRALIAN FEED SOURCES

Fungi are a normal part of the microflora of food and the species most often encountered belong primarily to five genera: *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Penicillium*. Other genera including *Chaetomium*, *Claviceps*, *Diplodia*, *Myrothecium*, *Phoma*, *Omopsis*, *Pithomyces* and *Stachybotrys* are often contaminants of food. These moulds produce many different toxic compounds. Not all isolates of a toxigenic fungus produce toxins. The production of mycotoxins depends on the fungus present, the composition of the feedstuff or substrate and the conditions of handling and storage, especially moisture and temperature. Moreover, mycotoxin build-up can occur in standing pastures and grains can become contaminated both before and after harvest.

All of the toxigenic moulds occur in Australia and documented cases of mycotoxicoses in local livestock are listed in Table 1. Of the disorders, aflatoxicosis, vulvo-vaginitis, feed refusal, leukoencephalomalacia, bovine hyperthermia and diplodiosis are conditions associated with intensively managed animals. The other maladies in Table 1 occurred in grazing animals.

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<table>
<thead>
<tr>
<th>Disorder</th>
<th>Species</th>
<th>Toxin</th>
<th>Source</th>
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<tr>
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<td>Broiler</td>
<td>Aflatoxin</td>
<td>Peanut meal</td>
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<td></td>
<td>Poult</td>
<td>Aflatoxin</td>
<td>Peanut meal</td>
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<td></td>
<td>Ewe</td>
<td>Aflatoxin*</td>
<td>Maize</td>
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<td>Sow</td>
<td>Aflatoxin</td>
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<td>Bread</td>
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<td>Calf</td>
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<td>Gilt</td>
<td>Zearalenone</td>
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<td>Gilt</td>
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<td>Gilt</td>
<td>Zearalenone</td>
<td>Maize</td>
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<td></td>
<td>Gilt</td>
<td>Zearalenone</td>
<td>Bread</td>
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<td>Feed refusal and vomiting</td>
<td>Pig</td>
<td>Deoxinvalenol</td>
<td>Wheat, triticale</td>
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<td>Leukoencephalomalacia*</td>
<td>Horse</td>
<td>Unidentified toxin</td>
<td>Maize</td>
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<tr>
<td>Bovine hyperthermia</td>
<td>Beef and dairy cattle</td>
<td>Ergot alkaloids</td>
<td>Ryegrass, contaminated barley, lupins &amp; wheat ryegrass pasture</td>
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<td></td>
<td>Grazing cattle</td>
<td>Ergot alkaloids</td>
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<tr>
<td>Lupinosis</td>
<td>Sheep</td>
<td>Phomopsin</td>
<td>Lupin stubble</td>
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<td>Cattle</td>
<td>Phomopsin</td>
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<td>Sheep</td>
<td>Phomopsin</td>
<td>Lupin seed</td>
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<td>Horse</td>
<td>Phomopsin</td>
<td>Lupin stubble</td>
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<td>Sporidesmin</td>
<td>Ryegrass</td>
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<td>Cattle</td>
<td>Sporidesmin</td>
<td>Ryegrass</td>
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<td>Geeldikkop</td>
<td>Sheep</td>
<td>Sporidesmin and unidentified toxin</td>
<td>Pasture</td>
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<td>Goat</td>
<td>Ergot alkaloids</td>
<td>Ryegrass</td>
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<td>Ergotism</td>
<td>Cattle</td>
<td>Ergot alkaloids</td>
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<td>Diplodiosi*</td>
<td>Cattle</td>
<td>Unidentified toxin</td>
<td>Maize cobs &amp; stubble</td>
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<td>Granulocytopena* and thrombocytopena</td>
<td>Cattle</td>
<td>–</td>
<td>Pasture</td>
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<td>Fescue foot</td>
<td>Cattle</td>
<td>Alkaloids*</td>
<td>Tall fescue</td>
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<td>Ryegrass stuggers</td>
<td>Sheep</td>
<td>Lolitrem</td>
<td>Ryegrass</td>
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<td>Paspalum stuggers</td>
<td>Sheep</td>
<td>Paspalinine</td>
<td>Paspalum</td>
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<td>Cattle Horse</td>
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* Disorder or mycotoxin suspected but not confirmed.
**Aflatoxin**

It was the discovery of aflatoxin as the cause of "Turkey X disease" in Great Britain in 1960 that prompted the world wide interest in toxigenic fungi. Bryden (1982) has reviewed the significance of aflatoxin in Australia. Aflatoxin has been shown to have caused toxicoses in poultry, sheep, pigs, cattle and dogs in Australia. Although the responsible fungi Aspergillus flavus and A. parasiticus are widespread in nature and local isolates are highly toxigenic, the climatic conditions and agronomic practices of this country suggest that this mycotoxin is not a major problem. However there is one exception to this general statement in that locally produced peanuts can have high levels of contamination especially in drought years (Graham, 1982).

It is now known that cyclopiazonic acid (CPA) is produced by many aflatoxigenic strains of *A. flavus*. Interestingly, there is now much evidence to suggest that the original outbreak of "Turkey X disease" may have been a combined toxicosis of aflatoxin and CPA (Cole, 1986a). The basis of the speculation is that both aflatoxin and CPA are required to reproduce "Turkey X disease" clinically. This toxin is also produced by species of *Penicillium*, and has been shown to be toxic to all farm and laboratory animals studied (Dorner et al. 1985), including sheep and laying hens (Cole et al. 1988). The toxin has a very immediate effect on egg shell quality when dosed at 5 to 10 mg/kg bodyweight. This effect may be due to the chelating properties of this tetramic acid. Another interesting feature of this toxin is that it is deposited in broiler meat (Norred et al. 1988). However there are no reports of surveys for this toxin in Australia.

**Zearalenone**

Hyperoestrogenism is a problem in pigs consuming diets, usually based on maize infected with *Fusarium graminearum* Group 2. Zearalenone is the responsible toxin and was determined in maize (1-8 mg/kg) in an outbreak of hyperoestrogenism in pigs in Queensland (Blaney et al. 1984). There were very early reports of the syndrome in Victoria (Pullar and Lerew, 1937) and a recent NSW report of the syndrome in pigs consuming mouldy bakery waste (B.M. Christie, pers. comm.) but no analysis for toxin was undertaken in these cases. It is likely that this toxin will be encountered from time to time as *F. graminearum* is widespread in wheat and maize growing regions of Australia—(Burgess et al. 1988).

**Deoxynivalenol**

In addition to zearalenone, *F. graminearum* Group 2 produces a number of other mycotoxins (Table 2). Deoxynivalenol (DON; vomitoxin) is a trichothecene which is toxic to pigs but much better tolerated by cattle and chickens. It has been found in locally grown wheat and triticale and associated with outbreaks of feed refusal and vomiting in pigs (Moore et al. 1985; Bryden et al. 1987b; Tobin, 1988). These authors reported levels of DON in feed ingredients of 0.6 to 34 mg/kg. There is much disagreement in the literature as to the dietary level of DON needed to cause feed refusal in pigs. This may reflect the analytical methods used, differences in deoxynivalenol tolerance amongst pigs or the presence of
unidentified toxins. Abbas et al. (1986) demonstrated 15-acetyl-deoxynivalenol in maize associated with feed refusal in pigs and contaminated with DON and zearalenone and suggested the 15-acetyl derivative of DON may play a role in the aetiology of feed refusal and emesis. Australian isolates of *F. graminearum* produce zearalenone, DON and nivalenol in culture (Blaney and Dodman, 1988).

Table 2  Mycotoxins produced by *Fusarium graminearum, Fusarium moniliforme* and *Fusarium equiseti*

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Mycotoxin</th>
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<tr>
<td><em>F. graminearum</em></td>
<td>3-acetyldeoxynivalenol, butenolide, deoxynivalenol, diacetoxyscirpenol,</td>
</tr>
<tr>
<td></td>
<td>diacetyldeoxynivalenol, fusarenon-X (4-acetyl-nivalenol), monacetoxy scirpenol, nivalenol, T-2 toxin, zearalenone</td>
</tr>
<tr>
<td><em>F. moniliforme</em></td>
<td>Deoxynivalenol, diacetoxyscirpenol, fusaric acid, fusarins (fusariogenins), fusariocins, gibberellins, moniliformin, T-2 toxin, zearalenone (FES or P-2 toxin)</td>
</tr>
<tr>
<td><em>F. equiseti</em></td>
<td>Acetoxy scirpenol (monodeacetylanguidin), butenolide, diacetoxyscirpenol (anguidin), equisetin, fusarenon-X, fusarochromanone, monacetoxy scirpenol, neosolan, nivalenol, diacetate, scirpenol, triacetoxyscirpenol, T-2 toxin, zearalenol, zearalenone</td>
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**Leukoencephalomalacia**

Equine leukoencephalomalacia (ELEM) has been recorded in various parts of the world, especially the United States, since last century. The disease occurs in horses and donkeys following the consumption of maize contaminated with *F. moniliforme*. It is a neurological disease that results in liquefactive necrosis in the white matter of one or both cerebral hemispheres and this lesion is considered pathognomonic for ELEM (Haschek and Haliburton 1986). Liver involvement is also seen in some horses. Pigs develop acute, massive pulmonary oedema, while sheep and cattle develop severe toxic nephrosis and hepatosis when dosed with toxic cultures of *F. moniliforme* (Haschek and Haliburton 1986). Corn screenings from an outbreak of ELEM caused hepatocellular carcinoma in rats after feeding for six months (Wilson et al. 1985). *F. moniliforme* produces a number of mycotoxins (Table 2) but it was only last year that the water soluble compounds, fumonisins, thought responsible for ELEM were isolated (Gelderblom et al. 1988). One of the compounds, fumonisin B₁, is believed to be carcinogenic.
Only one case of ELEM has been reported in Australia (Robertson-Smith et al. 1985) but the case was not confirmed. Nevertheless, the fungus is found throughout-eastern Australia (Burgess et al. 1988) and infects maize especially during wet seasons (Blaney et al. 1986). Isolates of *F. moniliforme* vary greatly in their toxicity (Marasas et al. 1984) but the toxicity of Australian isolates has not been reported.

**Diplodiosis**

Diplodiosis results from the ingestion of a neurotoxin produced by *Diplodia maydis* (Kellerman 1986). The syndrome involves lacrimation, salivation and ataxia, with muscle fasciculation, followed by a complete paralysis and death. The fungus is distributed worldwide, but except for one unconfirmed case in cattle grazing maize cobs and stubble in Queensland (Darvell 1964) the disease has only been reported in southern Africa (Kellerman, 1986).

**Granulocytopenia and thrombocytopenia**

Two Victorian outbreaks of this syndrome in Angus heifers (Nicholls et al. 1985) and dairy Shorthorn cows (Jeffers and Lenghaus 1986) resulted in high mortality. The cause of the disease has not been determined but the authors suggest that on all the available evidence the most likely aetiological agent is a fungal toxin. In many respects the disease is similar to stachybotryotoxicosis but toxigenic fungi were not isolated from hay or pasture associated with the outbreaks.

**Alternaria and Penicillium**

*Alternaria* is the most common contaminant of cereal grains in Australia (Klein 1987; Ali et al. 1989). Secondary metabolites of this fungus, namely alternariol (AOH) and alternariol monomethylether (AME), have been associated with poor production in broilers (Bryden et al. 1984) and laying hens (R. Fraser, pers. comm.). These two mycotoxins are considered to have such low toxicity in poultry (Bryden et al. 1985) that their presence would not explain the production drops observed. It has been demonstrated recently that the much more toxic compound, tenuazonic acid was a contaminant of the mouldy sorghum from the 1984 episode (S. Andrews and S. Lukas, pers. comm.). Other studies in the author's laboratory have demonstrated that many Australian isolates of *Alternaria* from wheat, barley and sorghum are toxic when assayed in a chick bioassay (Bryden et al. 1987a). This was not unexpected as *Alternaria* produces some thirty secondary metabolites (Watson 1984). Many of these compounds have not been tested for toxicity.

*Penicillium* is another genus that is widespread in nature and known to produce a variety of toxins including; penicillic acid, CPA, penitrem A, ochratoxin A, patulin and roquefortine (Mislivec 1981). Ochratoxin A is the causal agent of porcine nephropathy in Denmark and although ochratoxin has been found in Australian feed samples (Connole et al. 1981) it has not been associated with disease outbreaks in this country. Penitrem A is a tremorgenic mycotoxin that was assayed in a hamburger bun that had been moulded by *P. crustosum* (Hocking et al. 1988). A dog consuming the bun had developed severe muscle tremors and had great difficulty standing.

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Ergotism

Ergotism, referred to as "St Antons Fire" during the middle ages was attributed to witchcraft rather than the consumption of mould-contaminated food, was probably the first mycotoxicosis to be recognised (Van Rensburg and Altenkirk 1974). The term "ergot" is the common name given to species of the Claviceps fungi. There are a number of fungi in this genera that cause classical or gangrenous ergotism (C.purpurea), paspalum staggers, neurotoxic or convulsive ergotism (C.paspali), and bovine hyperthermia(C.purpurea). Ergot also specifically refers to the sclerotium formed by C.purpurea when it parasitizes the ovary of developing grass flowers.

C.purpurea is widespread in Australia but there has been only one report of classical ergotism. Fraser and Dorling (1983) described bilateral hindlimb lameness and gangrene in two Friesian heifers. The outbreak occurred in Western Australia during July after the heifers had consumed meadow hay containing perennial ryegrass seed heads containing the ergots of C.purpurea. In this disease, alkaloids contained within the sclerotium cause vasoconstriction of arterioles resulting in necrosis of extremities (nose, ears, tongue, tail and limbs) followed by dry gangrene.

Paspalum Staggers

Paspalum staggers is caused by tremorgenic mycotoxins (paspalitrems) contained within the sclerotium of C.paspali (Cole and Dorner 1986). Clinical signs of paspalum staggers are typical of staggers syndromes in cattle and sheep and include tremors, incoordination, hyperexcitability and in severe cases, ataxia. An affected animal may appear normal until disturbed. This syndrome has been reported in cattle, sheep and horses in this country for the last fifty years (Culvenor 1974).

Bovine hyperthermia

During January to April 1986, cattle in the Illawarra and Central Tableland areas of NSW were affected by an unidentified syndrome. The syndrome was characterized by hyperthermia evidenced by increased rectal temperature (41-42°C), increased respiration rate, and excessive salivation (Jessup et al. 1987). We were able to show that the diets being fed were contaminated with ryegrass that had been infected with C.purpurea (Burgess et al. 1986) and reproduce the syndrome in dairy cows (Jang et al. 1987) and Hereford steers (Ross et al. 1989) by adding ergot from field cases to experimental diets. There was no evidence of gangrene in any cattle. However feeding the ergot to chickens (Bakau and Bryden 1987) produced gangrene of the feet, the lesion most often associated with ergotism of C.purpurea. The field outbreaks with cattle occurred during summer and were exacerbated by daily temperatures in excess of 35°C but when chickens were fed ergot and subjected to similar temperatures no foot lesions developed. This appears to result from changes in blood flow to the legs of birds subjected to different temperatures while ingesting ergot (Bakau and Bryden 1987). Presumably the high ambient temperature counteracts the vasoconstriction caused by the ergotamine alkaloid component. Interestingly, cases of classical ergotism have only been reported during the cooler months of the year.
Lupinosis

Lupinosis is a mycotoxicosis that has been recorded in Australia for the last 20 years (Culvenor 1974) and is caused by the ingestion of toxins produced by the fungus Phomopsis leptostromiformis when it colonises dead lupin plants or stubble. It is recognised primarily as a disease of sheep, especially in Western Australia, but natural outbreaks have also been reported in cattle, goats, donkeys and horses (Allen 1987). The principal toxin, phomopsin A, is a linear, chloride-containing hexapeptide (Edgar et al. 1986) and the liver is the primary organ affected.

With the increased use of lupins in pig and poultry rations concern has been expressed as to the possibility of lupinosis developing in these species. On the basis of results reported by Allen et al. (1984) it is suggested that lupin seed 10% infected with Phomopsis can be safely fed to pigs. Higher levels of infection should be easily recognised visually and the infected lupins diluted or discarded. Apparently pigs are more tolerant to phomopsin ingestion than sheep (Allen et al. 1984).

Facial eczema and Geeldikkop

Like lupinosis, facial eczema (pithomyctotoxicosis) has occurred from time to time in sheep in southern Australia (Greenwood and Williamson 1985). Spores produced by the fungus Pithomyces chartarum contain the mycotoxin sporidesmin which causes the disease in animals ingesting herbage containing the spores. Spore counts in excess of $2 \times 10^7$ spores/g wet weight of pasture are considered toxic (Di Menna and Bailey 1973). The toxin causes inflammation, damage and occlusion of bile ducts. Consequently, degradation products of chlorophyll (phyllloerythrin) and bile are released into the general circulation, causing jaundice, photosensitisation, loss of production and in extreme cases death due to liver failure (Kellerman 1986).

Geeldikkop is a photosensitivity disease of sheep grazing South African pastures in which the annual herb Tribulus terrestris (caltrop, bindii) predominates. The same disease has been reported recently in sheep and goats in New South Wales and Western Australia (Glastonbury et al. 1984; Glastonbury and Boal 1985; Jacob and Peet 1987). The disease is a classical hepatogenous photosensitization of sheep and clinically is identical to facial eczema. Geeldikkop is more complex than facial eczema in that the animal must ingest both sporidesmin and T. terrestris (Kellerman 1986).

Fungal endophytes

Endophytes are fungi that spend all or nearly all of their life cycles within the host plant. Despite the widespread distribution of these fungi and knowledge of their existence since late last century it has been the discovery that endophytes in tall fescue and perennial ryegrass cause fescue summer toxicosis and fescue foot in cattle and perennial ryegrass staggers (PRGS) in sheep and cattle (Bacon et al. 1986; Siegel et al. 1987) respectively. Fescue summer toxicosis and fescue foot are associated with alkaloids produced by the endophyte. Lolitrems are the tremorgenic mycotoxins produced by the ryegrass endophyte that cause the neurological-disorder, PRGS. Both fescue foot and PRGS have been reported
in Australia for many years (Culvenor 1974). PRGS should not be confused with annual ryegrass toxicity in which bacteria carried by plant nematodes produce cornynetoxins in galls on the ryegrass (Culvenor and Jago 1985).

Implications from the field

Outlined above are 14 distinct mycotoxicoses, 7 of which were recorded in Australia during this decade for the first time. It is possible that field problems resulting from the ingestion of the responsible toxins have occurred previously and not been reported or gone undetected. The field cases described were acute, sporadic disease episodes that would have been obvious to the farmer and have disrupted farm income. However in many situations where mycotoxins cause insidious loss (e.g. non-descript product ion drops, illthrift, increased returns to first service, and reduced resistance to infectious pathogens) it will be very difficult to diagnose the underlying cause of the problem. It is likely that mycotoxins will be overlooked in the diagnosis of such cases. Bovine hyperthermia caused serious production drops on beef feed lots and in dairy herds but no mortalities. It was reported and thoroughly investigated because of the widespread nature of the problem. Had it been an isolated phenomena it is much less likely that it would have been investigated as extensively and subsequently reported.

For convenience mycotoxigenic fungi are often divided into two ecological groups: those that occur in crops prior to harvest and those that grow on commodities in storage. This is an arbitrary division because aflatoxin can be produced in feedstuffs by A. flavus both before and after harvest. However it can be a helpful approach when explaining or predicting mycotoxicoses. Of the mycotoxicoses described above all except some cases of aflatoxicosis and canine penitrem intoxication resulted from ingest ion of intoxicated pastures or grains infected by fungi prior to grazing or harvest, respectively. Moreover, as most fungi that attack plants prior to harvest are pathogenic, an appreciation of the epidemiology of these plant diseases in the Australian environment would greatly facilitate our understanding of the aetiology of the resulting mycotoxicoses.

New Zealand research has unravelled the aetiology of facial eczema (Smith 1987). Facial eczema is limited to warm temperate zones where stock are fed exclusively on pasture and particularly where they are grazed intensively. From this it can be appreciated why outbreaks of this disease occur in Victoria, N.S.W. and Western Australia but not in the cooler climate of Tasmania. From an understanding of the epidemiology of the fungus, P. chartarum, in conjunction with pasture spores counts it is possible to forecast an outbreak of the disease and take evasive action.

The epidemiology of other fungal diseases is not as well understood but an extensive survey by Burgess and his co-workers (Burgess et al. 1988) of the distribution of Fusarium in eastern Australia soils coupled with an understanding of the ecology and climatic requirements of these fungi (Burgess 1981; Blaney 1985) provides an explanation of the occurrence and distribution of the Fusarium toxicoses described above. Although toxigenic Fusarium can be isolated throughout eastern Australia it is only in certain areas of Queensland and the far north coast of NSW that
the combination of agronomic practices and climatic conditions are such to favour mycotoxin formation from these fungi in the field. It was in these areas that the cases of hyperoestrogenism and feed refusal and vomiting were diagnosed.' However in their survey, Burgess et al. (1988) did isolate some 15 species of Fusarium many of which are known to be highly toxigenic. F. crookwellense, for example, was first described after being isolated near Crookwell, N.S.W. (Burgess et al. 1982) but the toxicity of local isolates is not known. Isolates of this fungus from South Africa (van Wyk et al. 1986), Poland (Golinski et al. 1988) and New Zealand (Lauren et al. 1988) have demonstrated high toxigenicity. It produces zearalenone, butenolide and trichothecenes. The production of zearalenone by this and other species of Fusarium on pasture has been suggested as a possible factor in the aetiology of reproductive problems of sheep in some areas (Lauren et al. 1988). Another Fusarium that is widely distributed and produces an array of toxins is F. equiseti. Fumarochromanone, produced by this fungus, induces tibial dyschondroplasia (TD) in chickens and has been suggested as an aetiological agent in this bone disorder of broiler chickens and turkeys (Walser 1987). The significance of this toxin in field cases of TD has not been elucidated.

Although Fusarium toxins have been responsible for diseases in Australian livestock the most common contaminant of locally grown grain is Alternaria (Klein 1987; Ali et al. 1989). This fungus also produces an array of toxins, many of which are difficult to analyse and their toxicity in livestock has not been evaluated. It is likely that reports of field cases of Alternaria mycotoxicoses will increase as analytical techniques improve and details of the effects of these toxins are described.

It is probable that many field toxicoses from ingestion of mycotoxins of Fusarium and Alternaria result from simultaneous ingestion of low levels of a number of toxins. In such instances, diagnosis based on symptoms derived from laboratory studies, evaluating single toxins, may have little resemblance to field outbreaks (Hamilton 1978). Moreover, most laboratory studies examine acute intoxication while most field cases involve low level chronic intoxication.

The discussion above has focussed on the involvement of moulded grains as the vehicle for mycotoxins to enter the feed supply but other significant feed ingredients include oilseed meals and various varieties of peas and beans. Overseas research has shown these commodities to have a varied mycoflora, many species of which are toxigenic. Cottonseed meal, for example, has been implicated in a number of outbreaks of aflatoxicosis in U.S.A. dairy herds (Applebaum et al. 1982), toxigenic fungi have been isolated from apparently healthy sunflower seeds (Vijayalakshmi and Rao 1985) and trichothecenes and zearalenone have been assayed in soyabees (Richardson et al. 1985). However, if the mycoflora of these feed commodities has been studied in the Australian environment it has not been reported. The one exception is peanut meal which has been associated with cases of aflatoxicosis (Bryden 1982).

Finally, the importance of fungal endophytes in animal toxicoses is just beginning to be appreciated. In addition to their presence in species of Festuca and Lolium, they have also been isolated from species of Bromus (White and Cole 1986), Poa (White 1987) and Stipa (White and Morgan-Jones 1989).
These studies suggest that the impact of endophytic fungi on grassland throughout the world could be considerable but there has been little research on their significance in Australia. There are a number of poorly understood syndromes of grazing cattle including kikuyu poisoning (Wong et al. 1987), oats sickness (S. Jagoe and B. Adams, pers. comm.) liver necrosis syndrome and Stewards Range tremor syndrome (M. Hindmarsh, pers. comm.) that are "plant" toxicosis but the toxin and its origin, whether it be plant or perhaps a fungal endophyte, remains to be delineated.

**MYCOTOXIN ANALYSIS**

Within the present context we are primarily concerned with screening for mycotoxins in feedstuffs. Bioassays have been the traditional approach to this problem and still play an important role in delineating field toxicoses and complementing chemical techniques in the initial detection and isolation of unknown mycotoxins (Cole et al. 1986).

A major difficulty in analysis of feed is that of obtaining a representative feed sample. This is often difficult because of the existence of "hot spots" of fungal proliferation and the resulting uneven distribution of toxin within the suspect feed. Moreover, production problems may become apparent too late for a sample to be obtained as the feed may have already been consumed (e.g. a drop in egg production may not occur until a few days after aflatoxin has been eaten). It should not be surprising, therefore, that feed sampling is the greatest source of error in attempts to confirm a mycotoxicosis (Davis et al. 1980). Another major difficulty in analysis is the vast array of chemical compounds that constitute mycotoxins. Detection of many of these compounds requires very sophisticated and expensive laboratory equipment and very skilled analytical chemists. These techniques (Table 3) have recently been reviewed in an excellent monograph, (Cole 1986b). The reader is referred to this text for a complete discussion of these methodologies.

Application of immunological methods to mycotoxin analysis is a most exciting advance in mycotoxin detection and has been reviewed by Chu (1984, 1986). Immunoassays appear to offer the best opportunity for the development of rapid, repeatable and sensitive assays. These techniques are based on the highly specific reaction between an antibody and antigen and require the development of an antibody against the mycotoxin that is to be assayed. Mycotoxins are non-antigenic but are able to illicit an antibody response after conjugation to a protein or polypeptide carrier. The availability of antibodies to a number of mycotoxins has allowed the development of radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (ELISA) for the detection of toxins in feedstuffs and residual mycotoxins or metabolites in body fluids. The ELISA technique offers advantages over RIA in that radioisotopes and appropriate monitoring equipment are not required. It is also more adaptable to field use and commercial aflatoxin ELISA kits have recently become available.

The improvement and extensive use of ELISA procedures for mycotoxin analysis depends on the availability of antibodies. The application of biotechnology, namely monoclonal antibody or hybridoma techniques will facilitate the application of ELISA. Monoclonal antibodies against aflatoxin (Woychick et al. 1984), ochratoxin (Rousseau et al. 1987)
Zearalenone (Dixon et al. 1987) have been produced.

Table 3. Analytical methods for mycotoxin analysis

<table>
<thead>
<tr>
<th>Method</th>
<th>Mycotoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin-layer chromatography (TLC)</td>
<td>Aflatoxins, ochratoxins citrinin, sterigmatocystin, zearalenone, trichotheccenes, patulin, penicillic acid.</td>
</tr>
<tr>
<td>Gas chromatography (GC)</td>
<td>Trichotheccenes, zearalenone, patulin, penicillic acid, slaframine, swainsonine, Alternaria toxins, aflatoxins.</td>
</tr>
<tr>
<td>High-performance liquid chromosomeography (HPLC)</td>
<td>Aflatoxins, ochratoxins, zearalenone, trichotheccenes, moniliformin, patulin, ergot alkaloids, penicillic acid, PR toxin, Alternaria toxins.</td>
</tr>
<tr>
<td>Gas chromatographic - Mass spectrometric analysis (GC-MS)</td>
<td>Trichotheccenes, zearalenone, aflatoxins, patulin, sterigmatocystin.</td>
</tr>
<tr>
<td>Mass spectrometry - Mass spectrometry or Tandem Mass spectrometry (MS-MS)</td>
<td>Ergot alkaloids, zearalenone, trichotheccenes, aflatoxins, roridins.</td>
</tr>
<tr>
<td>Immunoassays</td>
<td>Aflatoxins, trichotheccenes, zearalenone, ochratoxins, sterigmatocystin.</td>
</tr>
</tbody>
</table>

CONTROL OF MYCOTOXINS IN FEEDS

Feedstuffs can become contaminated with mycotoxins prior to, during, or after harvest, during processing or while in farm storage prior to consumption (Howell 1983).

All stockfeeds serve as a suitable substrate for mould growth and mycotoxin formation. In addition to the genetic capacity of the fungus, mycotoxin production depends on many factors which have been reviewed in great detail by Bullerman et al. (1984). Moisture and temperature are two factors that have a crucial effect on fungal proliferation and toxin elaboration. The minimum critical levels for the growth of fungi are 7-15 per cent moisture and 80-85 per cent relative humidity. Temperatures at which toxin production can take place vary from 0°C to 35°C, depending on the fungal species. However, conditions that favour maximum fungal growth may not be conducive to mycotoxin production by the fungus.
As mycotoxin can be elaborated in feed ingredients (e.g. cereal grains) prior to harvest preventive measures begins with good agronomic practices including cultivating to improve plant vigour, the judicious use of insecticides and fungicides to reduce insect and fungal infestation, irrigation to avoid drought conditions, harvesting at maturity and, more recently, breeding programmes to improve genetic resistance to fungal attack.

After harvest, control of moisture and temperature of the stored commodity will largely determine the degree of fungal activity (Northolt and Bullerman 1982). Moisture depends mostly on water content at harvest, the amount of drying, aerating, and turning of the grain before or during storage as well as the respiration of insects and microorganisms. Rewetting may occur by leaks or flooding into storage units, but by far the most common cause is condensation which can give rise to the development of "hot spots". Condensation often results from moisture migration in bulk stores under the influence of temperature differentials within the grain that are mediated by fluctuations in ambient temperature. These problems, are minimised in compounded feeds if storage time is kept to a minimum (Good and Hamilton 1981).

Apart from methods that modify the fungal environment many compounds are available that will inhibit mould growth in feedstuffs. These have been reviewed by Ray and Bullerman (1982). Organic acids, especially proprionic acid form the basis of many commercial antifungal agents used in the stockfeed industry and give excellent protection. However, their effectiveness depends on a number of factors related to the feed to which they are added. These have been reviewed by Hamilton (1985).

Once a mycotoxin has been formed in a feed it is difficult to reduce its concentration because of the stability of these compounds. The obvious approach is dilution with uncontaminated feed. Where this is not practical various techniques have been proposed. Doyle et al. (1982) have reviewed the physical, chemical and biological means of mycotoxin degradation and concluded that, "no single treatment has proven completely successful in degrading or removing toxins and retaining the nutritional and functional qualities of the treated commodity". Despite these reservations, ammoniation is now used commercially to destroy aflatoxin in peanuts (Prevolt 1986) and bisulfite shows promise for destruction of deoxynivalenol in grains (Young et al. 1987). Other compounds including, activated charcoal and bentonite, significantly reduce the gut absorption of some mycotoxins contained in feed by adsorption of the toxin.

CONCLUDING REMARKS

The insidious nature of many mycotoxicoses make it virtually impossible to estimate their incidence and economic impact. If one considers the various aspects of loss from a mycotoxicosis, one is faced with a multitude of possibilities. Obviously, they are complex and deal with many aspects of animal production especially during chronic intoxication. Losses due to death are easy to determine but losses due to morbidity are not easily analysed yet they may be of greater economic significance. Many factors would have to be considered in attempting to determine economic loss: epidemiological surveys including clinical and
laboratory procedures; loss of contaminated feed and cleaning of contaminated feeding equipment; incorporation of antifungal agents into feedstuffs; reduction in animal productivity, increased mortality, and predilection of animals and birds ingesting mycotoxins, to infection by secondary pathogens. To this list might be added the growing forensic aspect of mycotoxicoses. A Canadian court decision in favour of a pig producer against a feed company (Schiefer and O'Ferrall 1981) highlights the problems associated with this aspect of fungal infected feed. Similar cases have been listed for hearing in Australian courts but have been settled before court appearances were made.

REFERENCES


